Colloidal Drug Probe: Method Development and Validation for Adhesion Force Measurement Using Atomic Force Microscopy

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Abstract — This study demonstrates a novel technique of preparing drug colloid probes to determine the adhesion force between the drug salbutamol sulphate (SS) and the surfaces of polymer microparticles to be used as carriers for the dispersion of drug particles from a dry powder inhaler (DPI) formulation. Initially model silica probes of approximately 4 µm size, similar to a drug particle used in DPI formulations, were coated with a saturated SS solution with the aid of capillary forces acting between the silica probe and the drug solution. The developed method of ensuring a smooth and uniform layer of SS on the silica probe was validated using X-Ray Photoelectron Spectroscopy (XPS) and Scanning Electron Microscopy (SEM). Using the same technique, silica microspheres preattached on the AFM cantilever were coated with SS. The adhesion forces between the silica probe and drug coated silica (drug probe) and polymer surfaces (hydrophilic and hydrophobic) were determined. Our experimental results showed that the technique for preparing the drug probe was robust and can be used to determine the adhesion force between hydrophilic/hydrophobic drug probe and carrier surfaces to gain a better understanding on drug carrier adhesion forces in DPI formulations.

Keywords- Atomic Force Microscopy, Adhesion Force, Coating, Silica probe, Validation

I. INTRODUCTION

Dry powder inhaler (DPI) formulations are an interactive mixture of micronized drug particles (<5µm) adhered on the surface of large carriers from where drug particles need to be detached for deep lung delivery. The interaction forces between drug-drug, drug-carrier and drug-inhaler walls are important factors that control the efficient dispersion of drug from the formulation.

The emergence of atomic force microscopy (AFM) has enabled researchers to directly measure the adhesion forces between the surfaces of different particles. Ducker *et al*, the pioneers in using AFM colloid probe for studying the adhesional properties between two surfaces, measured the particle adhesion forces by mounting a silica sphere on the AFM tip (Ducker *et al.*, 1991). Various combinations of drugs and carriers have been studied using the Ducker *et al* technique including lactose and salbutamol sulphate and budesonide (Price et al., 2002), salbutamol sulphate particle and compacted salbutamol sulphate surface (Young et al., 2003a), micronized zanamivir and lactose surface (Berard *et al.*, 2002a, 2002b), and salmeterol xinafoate and lactose (Islam *et al.*, 2005). While the accurate measurement of adhesion forces between particles in DPI formulations is technically challenging it is important for understanding and optimizing efficient delivery of drug from the formulations.

In order to measure the adhesion forces between particles, the AFM colloid probe is prepared by a traditional method (Ducker et al., 1991; Eve et al., 2002; Islam et al., 2005; Tsukada et al., 2004): a silica glass bead of <5µm size (simulating the size of inhalable drug particle used in DPI formulation), used as a colloid particle is mounted near the apex of the cantilever using a minute amount of epoxy resin by means of a 3D micromanipulator. The technique has been illustrated in Figure 1. Using a micromanipulator a piece of tungsten wire, clamped in the micromanipulator, is dipped into a small amount of pre-cured epoxy resin. This process is observed through an optical microscope. After withdrawal from the resin, the tip of the wire is carefully brought in contact with a microscope slide to remove excess resin from the wire tip. The resin coated wire tip is then brought into contact with the apex of the silicon nitride cantilever. It is important to transfer only a small amount of resin on the cantilever tip to prevent the immersion of the particle in the

wet resin. After cutting the glued wire tip, it is brought into contact with a silica sphere which adheres to the wire tip due to capillary forces (Figure 1).

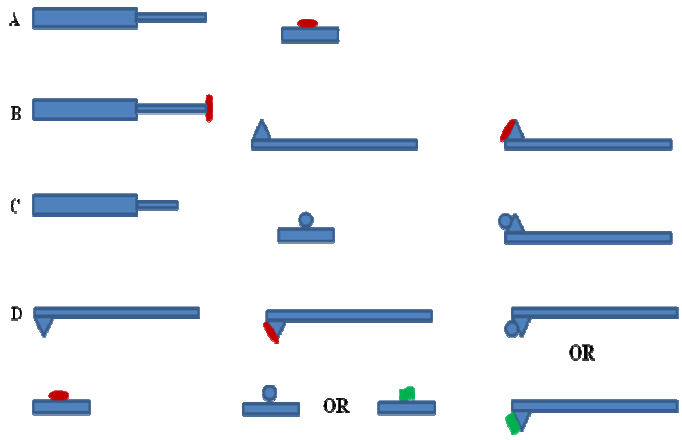

Figure 1. Traditional method for preparation of AFM colloid probe
A: A piece of tungsten wire, clamped in the micromanipulator approaches towards the epoxy resin. The excess resin from the wire tip is removed by contacting the wire with a glass slide.
B: Resin coated wire tip approaches to the apex of cantilever and a small amount of resin is transferred to the cantilever.
C: The glued wire tip is cut and removed; a freshly cleaned wire is brought into contact with a silica sphere, which is then attached to the cantilever tip where it adheres with the help of previously transferred resin film.
D: Epoxy resin is attached on the tip of the cantilever by the process described above. The cantilever is positioned above the single drug particle or drug agglomerates and lowered microscopically until contact with the drug particle occurs. The cantilever is retracted so that the drug particles are transferred to the apex of the cantilever for force measurements. Excess drug particles are removed by gently blowing the cantilever with nitrogen. All steps are monitored under a microscope.

The sphere is then carefully transferred to the cantilever tip where it adheres to the resin. Great care must be taken so that epoxy resin does not spread around the sphere. The difficulty arises with the attachment of a single drug particle onto the tip because drug particles less than 5 microns are highly cohesive and form agglomerates. Using this method, epoxy resin is applied to a cantilever tip, which is positioned in the AFM for the force measurements. It is positioned directly above a single drug particle, or a tiny agglomerate of drug particles, and lowered microscopically until contact occurs. Retraction of the tip enables initial confirmation of particle attachment. After drying, the drug probe is examined under an optical microscope fitted with a camera to ensure successful attachment. Excess drug particles must be removed by blowing with nitrogen and finally the size of the colloid particle is determined via microscopy. Although the attachment of many drug particles onto the AFM tips has been studied using this technique , due to the geometry (uneven and non-spherical shape) of the drug particle, the measured adhesion force may not agree with theoretically predicted values. Moreover, isolation and attachment of a single drug particle with the required size (<5 µm), which are highly cohesive and formed agglomerates is very difficult.

The successful attachment of colloid probes (silica or drug particles) on the AFM tip is finicky, time consuming and highly dependent on operator skill. To represent the exact drug probe with spherical shape, chemical modification of the surface of colloid probes and their attachment of AFM tips has been demonstrated (Xu et al., 2006). For example, to measure the hydrophobic force, both the hydrophilic colloidal silica probe and the substrate surface are modified to be hydrophobic by molecular self-assembly technology (Rabinovich and Yoon, 1994). For a single silica sphere, the pre-cleaning treatments, chemical modification and final cleaning procedure after the modification is not straightforward. Additionally, the attachment of surface modified colloids on the cantilever tips is strenuous and there is a possibility of changing the surface during attachment using a micromanipulator. Xu *et al* developed a chemical etching technique to prepare a colloid probe for measuring the hydrophobic forces by AFM; however, a disadvantage of this technique is that the colloid chemical modification, etching and cleaning processes are very time consuming (Xu et al., 2006). Recently, Li *et al* modified an AFM tip with polymers (chitosan and PLGA) to study the interactions between polymer and mucin film (Li et al., 2010). The authors used an AFM tip coated with a thin layer of polymer. We believe this limits its applicability because the measured adhesion force cannot be compared with JKR and DMT theory due to the irregular geometry of the tip (i.e., diameter of polymer coated AFM tip is not certain). In addition, tip wear is another matter of concern as it affects the force measurement which results in decreased accuracy and reproducibility of measurements (Haochih Liu and Chen, 2011). Surface roughness of both drug particles and drug and carrier particles affect the adhesion and detachment force and thus the traditional method of attaching a single drug probe for force measurement is not necessarily representative.

In this study, we have developed a method of preparing colloidal SS probe for adhesion force measurement, which potentially overcomes the shortcomings of the traditional methods and contributes to the improvement of the surface force measurements by AFM. Using this novel technique, the drug (SS) colloid probe was prepared, validated and the adhesion force between the drug probe and polymer carrier surfaces was determined at ambient conditions.

II. EXPERIMENTAL

A. *Chemicals*

Micronized Salbutamol Sulfate (SS) of inhalation grade (Volume Median Diameter: 5µm) was obtained from GlaxoSmithKline, Australia. Polycaprolactone (PCL) (M_W 80,000), polyvinyl alcohol (PVA) (87-89% hydrolyzed, M_W 85,000-124,000) and L-Leucine were from Sigma Aldrich. Magnesium stearate (MgSt) was obtained from PCCA, Australia. Dichloromethane (DCM) was of analytical grade.

B. *Preparation of SS probe*

The silica sphere on the cantilever was functionalized with SS solution to act as a SS probe. The drug was coated on the silica sphere of the cantilever with the aid of approach/retract cycle of AFM scanner. A small drop of supersaturated solution (5%w/v) of SS was placed on a clean glass slide, which was placed on the scanner of the AFM. The silica probe (pre attached on the cantilever tip, CP-FM-SiO-B-5, NanoAnd More, Germany) to be functionalized with SS was secured on the cantilever holder and positioned exactly above the SS solution. The cantilever, made to approach to the drug solution on the surface using the microscope's feedback loop with a controlled motion, was kept immersed in the SS solution for 10 minutes; retracted from the solution after 10 minutes and allowed to dry for a period of 30 minutes. This resulted in the formation of multilayered coating of SS on the silica sphere. This method ensured a SS layer on the spherical silica probe, suitable for adhesion force measurements (Figure 2).

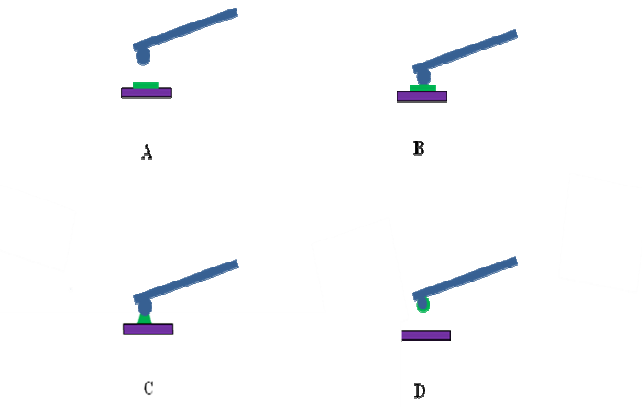

Figure 2. Preparation of colloidal drug probe
A: A small drop of supersaturated drug solution is placed on a clean glass slide below the silica sphere preattached to the cantilever
B: The cantilever approaches the drug solution until it touches the surface of the solution at a controlled motion using the feedback loop of the AFM scanner
C: The cantilever is kept immersed in the drug solution for 10 minutes, retracted from the solution and allowed to dry for 30 minutes
D: A multilayer coating of drug is formed on the surface of the silica sphere

C. *Measurement of Adhesion force*

The adhesion forces of the polymer films with the uncoated and coated silica sphere were determined by AFM using a colloid probe technique. The silicon nitride cantilever (225 ± 10 µm long, 28 ± 7.5 µm wide and 3 ± 1 µm thick) used for the determination of adhesion forces had a single silica particle (sphere of diameter 3.5 ± 0.1 µm) preattached on the tip of the cantilever. The cantilevers have resonance frequencies ranging from 45-115 kHz and force constants ranging from 0.5-9.5 N/m. The spring constants (k) for each cantilever were determined using the thermal noise method (Hutter and Bechhoefer, 1993; Senden and Ducker, 1994). The spring constant was found to vary from 1.12 - 2.55 N/m before coating.

D. *Cleaning of glass wares*

Cover slips (22×22 mm) were used (Menzel Glaser, Germany) for the deposition of films. Harrick Plasma Cleaner (Model: PDC-002, Ithaca, NY) was used to eliminate possible contamination from the coverslips and to make them hydrophilic. The vacuum was attained with the help of dry oxygen service pump (Model XDS5, Edward, USA). Oxygen flow was kept at 0.1 SCFH (Standard Cubic Feet per Hour) using a plasma flow controller. The cleaning step consisted of 30 seconds oxygen plasma clean at 380 mTorr pressure and 29.6 W of discharge power at high RF level. Microscope glass slides (25.4 mm×76.2 mm×1 mm; Sail brand, China) were first washed with acetone and then with ethanol to remove the impurities and contaminants from the surface. Later the slides were blown with nitrogen to ensure complete drying.

E. *Preparation of polymer films*

Using a spin coater (Model PWM-32, Headway Research, Inc., Texas), a glass cover slip (used as a substrate) was coated with polymer solution (10% w/v PCL solution in DCM). The PCL film was formed by spinning the polymer solution at 1000 rpm for 20 seconds. Approximately, 1 mL of the polymer solution was placed on the centre of the cover slip and was spun at a constant speed until all the solvent was evaporated and a thin smooth film of polymer was formed on it. These samples were stored in a desiccator and later used for adhesion force measurements and imaging.

The PCL + PVA film (the PCL layer covered with PVA film) was formed by depositing the layer of PCL film as described above and the PCL layer was covered with a 1% PVA solution and spun at 2000 rpm for 60 seconds and allowing it to dry for 30 minutes. The 3 layer film of PCL + PVA + MgSt was prepared by depositing the first 2 layers in the similar manner as described above and later 1% MgSt solution was deposited and spun for 1000 rpm for 60 seconds. Similarly the 3 layered film of PCL + PVA + Leucine was prepared by depositing the first 2 layers as described above and later spinning 1% leucine solution at 5000 rpm for 60 seconds. A summary of the speed and the time interval used for each sample is provided in Table I.

Table I. Speed of the spin coater and time intervals of spinning of each sample

SAMPLE	SPEED (rpm)	TIME (s)
PCL	1000	20
PVA	2000	60
MgSt	1000	60
Leucine	5000	60

F. *Preparation of polymer microspheres*

The microparticles were prepared by oil in water (o/w) solvent evaporation method. The polymer PCL was dissolved in dichloromethane (DCM) at 10% concentration. This polymer solution was added dropwise into 1% w/v aqueous polyvinyl alcohol (PVA) solution with the aid of a dropping funnel. The emulsion was stirred with an overhead stirrer (IKA® RW 20

digital Labtek, Model RW20D) at a constant speed of 2000 rpm continuously for 40 minutes under ambient pressure and then stirred for another 20 minutes under reduced pressure on a rotary evaporator (Rotavapor R-210, BUCHI, Switzerland). Finally the microspheres were collected by filtration, washed with deionized water and dried in a vacuum desiccator at room temperature.

G. Force Measurement

All force measurements were performed using an AFM MFP-3D-BIO (Asylum Research, Technical Manufacturing Corporation, USA) and IGOR Pro 6.21 software (Wavemetrics, USA) in air and ambient conditions. Measurements in force volume mode were performed between an uncoated silica sphere and the film and microsphere, or an SS coated silica sphere and the film and microsphere. In the force volume mode, the AFM raster scans the substrate under the colloidal probe to produce a series of force curves, each from a well-defined location in the x and y directions. The results were produced as a force map that shows the variation in the forces of interaction in the defined area. For the films, the individual force curves were measured over a 10 µm × 10 µm area, at a scan rate of 1 Hz and a total of 8 × 8 (n=64) force points. The adhesion force determination was performed at 5 different spots on each film (n=5) and a total number of 320 force curves were measured for each film. For microspheres, the individual force curves were measured over a 10 µm × 10 µm area, at a scan rate of 1 Hz and a total of 32 × 32 (n=1024) force points. The adhesion force determination was performed on 5 different microspheres for each sample (n=5) and a total number of 5120 force curves were measured for each sample.

H. X-Ray Photoelectron Spectroscopy (XPS)

The surface composition of the PCL films coated with MgSt or leucine were analyzed by XPS. Samples were mounted onto stainless steel sample holders using double-sided adhesive tape. XPS analysis was performed with a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) equipped with monochromatized aluminium X-ray source (powered at 10mA and 15kV) and an eight-channeltron detector. The analyzed area was 800 × 200 µm. The constant pass energy was set at 160eV for the survey spectrum and 20eV for the multiplex spectrum. The following sequence of spectra was recorded: Survey spectrum, O1s, N1s, C1s, and Mg2p multiplex spectra. The elemental compositions of each material were determined using Casa XPS software Version 2.3.14.

I. Scanning Electron Microscopy (SEM)

For surface morphological studies of the silica particles, the samples were adhered onto aluminium stubs using double-sided carbon sticky tape. Particles were sputtered with gold (thickness ~ 15-20 nm) with a sputter coater (BIORAD SC-500 Sputter coater)) using an electrical current of 15 mA for 3 minutes. Several photomicrographs of the samples were taken at different magnifications using a Scanning Electron Microscope (FEI Quanta 200 SEM).

III. RESULTS AND DISCUSSIONS

A. Preparation and validation of SS probe for force measurement

This procedure for preparation of SS probe was first developed in our lab and was validated. The validation involved determining the time required by the silica sphere to be completely coated by the SS solution. Briefly, 100 mg of silica spheres were coated with the SS solution for periods of 5, 10 and 30 minutes. Later these samples were analyzed by XPS to detect the presence of sulfur (as SS is present as a sulfate salt) and silica. Initially the silica spheres coated for the time interval of 5 minutes showed the presence of sulfur in addition to the signal of some traces silica (XPS data, Figure 3B), which indicated the partial coating of silica sphere with SS. However, the spheres coated for the time interval of 10 and 30 minutes had a very strong signal of sulfur and silica was not found in the spectrum (Figure 3C and 3D) indicating that the spheres coated for 10 minute time interval was completely coated by SS. Hence the time period of 10 minutes was selected to functionalize the silica probe with SS. Figure 4 shows the SEM images of the uncoated silica sphere coated with SS at various time intervals. Figure 4C and D reveals that the silica sphere has been sufficiently covered with SS as compared with 4B is the scan of the sample coated for 5 minutes. An SEM image of the uncoated cantilever and cantilever functionalized with SS (drug probe) is shown in Figure 5.

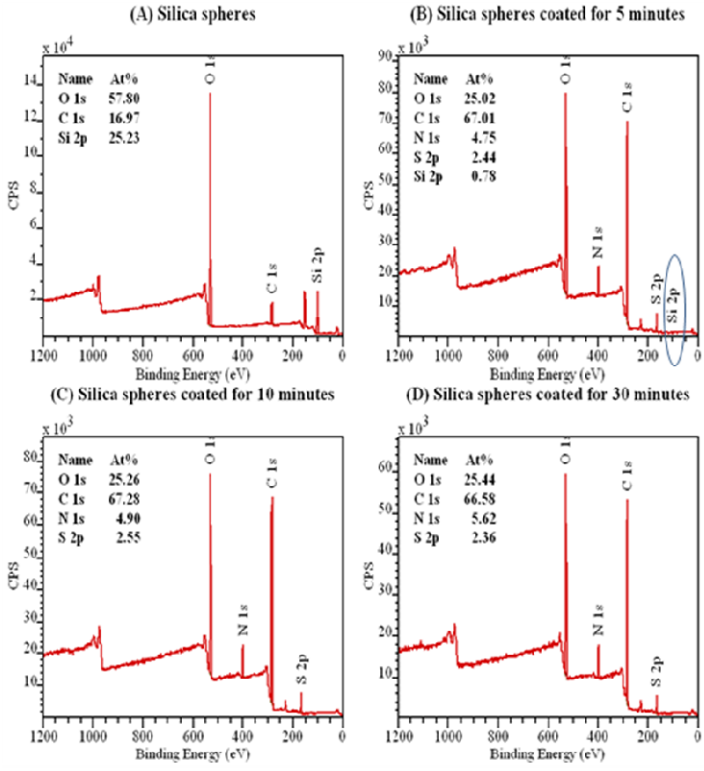

Figure 3. XPS survey scan of (A) Silica spheres (B) Silica spheres coated with SS for 5 minutes. These spheres in addition to sulphur peak also shows the presence of silica (Si2p) peak which indicates that partial coating of silica

spheres (C) Silica spheres coated with SS for 10 minutes and (D) Silica spheres coated with SS for 30 minutes

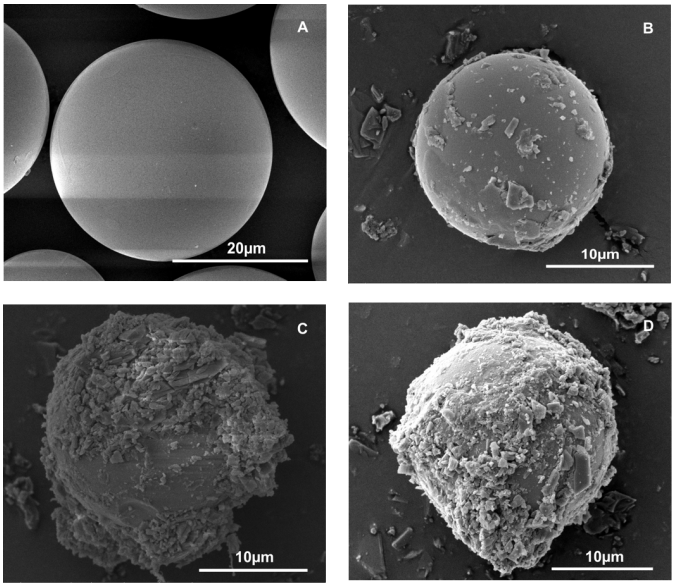

Figure 4. SEM images of (A) Silica sphere, (B) Silica sphere coated with SS for 5 min, (C) Silica sphere coated with SS for 10 min, and (D) Silica sphere coated with SS for 30 min

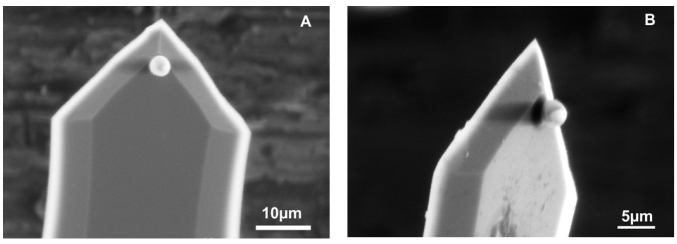

Figure 5. SEM image of (A) uncoated cantilever and (B) cantilever coated with SS

B. Validation of adhesion force measurement

The adhesion forces between the silica probe and the PCL microspheres were measured on three different microspheres at a time interval of 5 minutes each over a period of 30 minutes. The mean adhesion force and co-efficient of variance (CV) obtained for the three different PCL microspheres over a period of 30 minutes were 93.2 nN (0.7%), 135.4 nN (1.5%) and 167.3 nN (1.2%). Similarly the adhesion forces between the SS probe and the PCL microspheres were measured and the mean adhesion force and CV obtained for the three different PCL microspheres over a period of 30 minutes were 267.2 nN (0.1%), 298.8 nN (0.6%) and 326.4 nN (0.4%). The adhesion forces measured with drug-coated silica were much higher as compared to the uncoated silica sphere. This can be attributed to the size and geometry of the probes. After coating, the SS was crystallized on the silica sphere and the surface was not as smooth as prior to coating. The surface roughness of the drug-coated silica particles were not measured in this present study. The reproducibility of the adhesion forces between the silica probe and three different PCL microspheres and between the SS probe and PCL microspheres are shown in Figure 6 and 7, respectively. As demonstrated in these figures, the average forces between the respective probes and different surfaces are constant at different time interval. The low variability in the adhesion forces at each adhesion site confirmed that the adhesion forces were reproducible with very good stability.

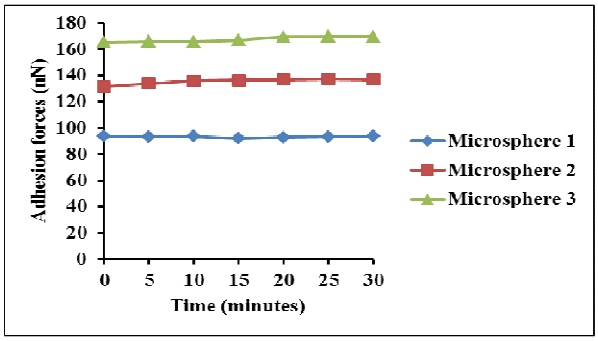

Figure 6. Adhesion forces measured at a particular site on three different PCL microspheres over a period of 30 min determined by AFM using 3.5 µm silica probe

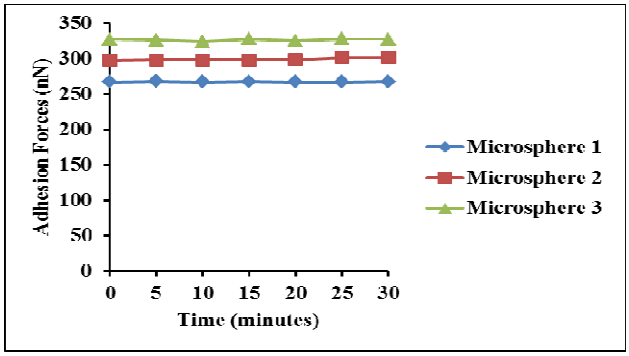

Figure 7. Adhesion forces measured at a particular site on three different PCL microspheres over a period of 30 min determined by AFM using 3.5 µm silica probe functionalized with SS

C. XPS analysis of films

Films of PCL were coated with PVA then MgSt or leucine and analyzed using XPS to detect the presence of coating materials on the surface of the polymer. The presence of the characteristic peak of magnesium (Mg2p) at 49.5eV (Figure 8A) and nitrogen (N1s) at 398eV (Figure 8B) confirmed that the films had been successfully coated with MgSt and leucine solutions, respectively.

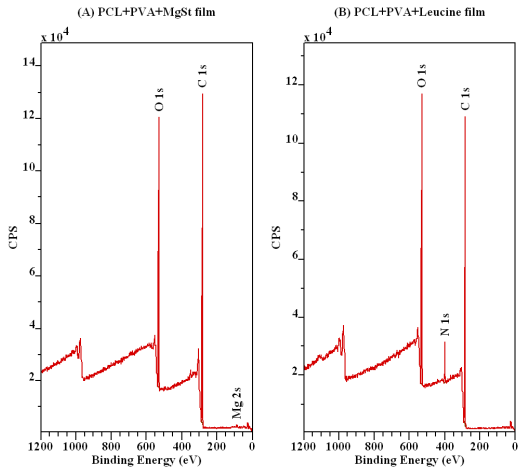

Figure 8. Survey spectra of (A) three layered film of PCL, PVA and MgSt and (B) three layered film of PCL, PVA and Leucine

D. Adhesion force of films

After the validation of SS probe preparation and adhesion force measurement, this technique was applied to investigate the adhesion forces between hydrophilic probes (both silica and SS probe) and polymer surfaces. It was found that the adhesion forces between the silica probe and uncoated PCL film was high (153.7 ± 7.0 nN). The hydrophobic PCL film was rendered hydrophilic by depositing a second layer of PVA on the surface of PCL which is called as PCL+PVA film. The adhesion force between silica probe and PCL+PVA film dropped to 103.7 ± 8.4 nN. Further deposition of a third layer of MgSt or leucine on the surface of bilayer films of PCL+PVA led to formation of PCL+PVA+MgSt or PCL+PVA+Leucine film and the adhesion force drastically reduced to 56.9 ± 6.0 nN and 60.3 ± 8.5 nN for 1% and 2% PCL+PVA+MgSt films, respectively and to 73.7 ± 5.1 nN and 77.8 ± 4.8 nN for 1% and 2% PCL+PVA+Leucine film, respectively (Table II). It was observed that the uncoated PCL film exhibited high adhesion forces with the silica sphere and this force was reduced in the presence of MgSt and leucine coatings on the PCL surface (Figure 9). Both, MgSt and leucine are hydrophobic and the surface of PCL was covered with these hydrophobic excipients. Thus the reduction of adhesion forces was considered due to the hydrophobic nature of the surfaces.

The adhesion force between SS probe and the uncoated PCL film was found to be 776.1 ± 26.9 nN and with the PCL+PVA film was 295.6 ± 26.2 nN. When the adhesion forces were determined between SS and three-layered film of PCL+PVA+1% or 2% MgSt, the forces of interaction declined to 131.3 ± 14.7 nN and 139.1 ± 25.3 nN, respectively ($p<0.05$, n=5). Similarly the forces between SS and 1% or 2% leucine coated PCL+PVA films were reduced to 137.1 ± 24.5 nN and 153.1 ± 23.8 nN, respectively ($p<0.05$, n=5) (Table II). There were no significant differences ($p>0.05$, n=5) in the forces between the two concentrations (1% or 2%) in either cases of MgSt or leucine (Figure 10). The adhesion forces between SS probe and PCL polymer were very high and upon deposition of PVA on the surface of PCL, the forces of interaction were decreased between PCL+PVA and SS. This could be due to the presence of certain functional groups of PVA on the surface of the PCL polymer which could have led to strong interaction between the drug and the polymer. Further deposition of MgSt and leucine on the surface of PCL+PVA decreased the adhesion forces with SS by forming a hydrophobic film on the surface. This data indicated higher interaction force between PCL polymer and SS compared to the adhesion forces between SS and PCL+PVA, PCL+PVA+MgSt, and PCL+PVA+Leucine. Thus, the PCL without coating exhibited the highest adhesion forces and the addition of MgSt and leucine which acts as anti-adherent aided in the reduction of adhesion forces.

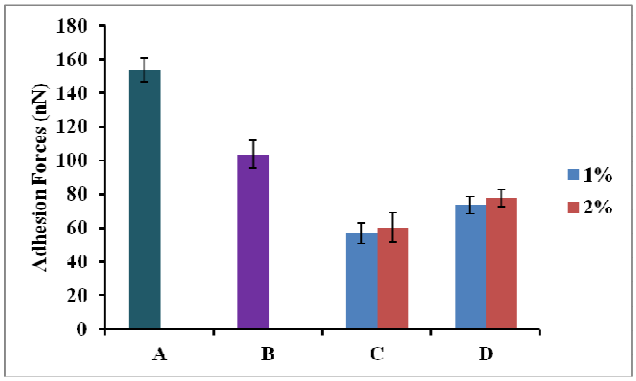

Figure 9. Adhesion forces between uncoated silica sphere and PCL film, n=5. A: PCL film; B: PCL+PVA film; C: PCL+PVA+MgSt film; D: PCL+PVA+Leucine film.
The error bars correspond to the standard deviation.

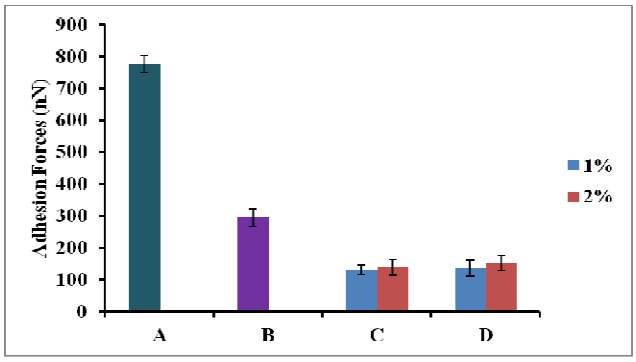

Figure 10. Forces between SS coated silica sphere and PCL films, n=5. A: PCL film; B: PCL+PVA film; C: PCL+PVA+MgSt film; D: PCL+PVA+Leucine film. The error bars correspond to the standard deviation.

Table II. Adhesion force measurements of PCL and coated PCL films with uncoated and coated silica sphere, n=5

Sample	Adhesion forces (nN)	
	Uncoated Silica sphere	Silica sphere coated with SS
PCL	153.7 ± 7.0	776.1 ± 26.9
PCL+ PVA	103.7 ± 8.4	295.6 ± 26.2
PCL+ PVA+ MgSt (1%)	56.9 ± 6.0	131.3 ± 14.7
PCL+ PVA+ MgSt (2%)	60.3 ± 8.5	139.1 ± 25.3
PCL+ PVA+ leucine (1%)	73.7 ± 5.1	137.1 ± 24.5
PCL+ PVA+ leucine (2%)	77.8 ± 4.8	153.1 ± 23.8

E. Adhesion force of microspheres

The adhesion forces between the silica or drug probes and polymer microspheres which are representative of drug and carrier in the DPI formulation have been investigated. The force between the uncoated silica sphere and PCL microspheres was found to be 150.1 ± 15.5 nN. When the adhesion forces were determined between the uncoated silica sphere and 1% MgSt coated PCL microspheres, the forces of interaction were found to decrease from 150.1 ± 15.5 nN to 73.3 ± 16.3 nN ($p<0.05$, n=5). Similarly the adhesion forces between the uncoated silica sphere and 1% leucine coated PCL microspheres were found to reduce to 81.6 ± 16.6 nN ($p<0.05$, n=5) (Figure 11). The adhesion forces between the drug probe and the PCL microspheres have been demonstrated in our previously published paper (Tuli et al., 2012) where the adhesion force between the silica sphere functionalized with SS (drug probe) and PCL microspheres was 301.4 ± 21.7 nN (Table III). The forces between SS probe and 1% MgSt coated PCL microspheres drastically reduced to 110.9 ± 30.5 nN ($p<0.05$, n=5). Likewise, the adhesion forces between SS and 1% leucine coated PCL microspheres was reduced to 148.1 ± 21.0 nN ($p<0.05$, n=5) (Table III).

Thus in the case of microspheres, higher adhesion forces were exhibited with PCL polymer which was reduced in the presence of MgSt and leucine coatings due to their anti-adherent property consistent with the findings using PCL films. Therefore, the SS probe developed by our lab has been successfully applicable in studying the adhesional properties between hydrophilic-hydrophilic and hydrophilic-hydrophobic surfaces.

Table III. Adhesion force measurements of PCL and coated PCL microspheres with uncoated and coated silica sphere, n=5

Sample	Adhesion forces (nN)	
	Uncoated Silica sphere	Silica sphere coated with SS
PCL microspheres	150.1 ± 15.5	301.4 ± 21.7
PCL microspheres coated with 1% MgSt solution	73.3 ± 16.3	110.9 ± 30.5
PCL microspheres coated with 1% leucine solution	81.6 ± 16.6	148.1 ± 21.0

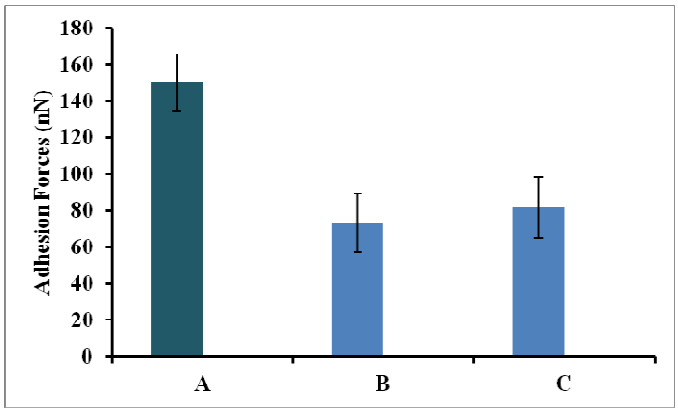

Figure 11. Adhesion forces between the SS probe and PCL films, n=5. A: PCL microsphere; B: PCL+MgSt (1%) microsphere; D: PCL+Leucine (1%) microsphere. The error bars correspond to the standard deviation.

CONCLUSIONS

A robust method for preparing drug colloid probe for AFM studies was developed and validated. Using this technique, we have successfully modified the AFM silica probe with SS solution. Adhesion forces between developed drug probe and polymer surfaces of hydrophilic and hydrophobic nature were determined in air. The observed force measurement can be explained with the surface hydrophilic/hydrophobic characteristics of the drug probe and polymer surfaces. Based on our experimental results the accurate interactions between a drug particle and carrier surface in DPI formulation can be determined. This unique method of preparing drug probe may be useful to get better understanding of the adhesion forces between a drug and carrier in DPI formulation.

ACKNOWLEDGMENTS

This work has been financially supported through PhD studentship and Rinku Tuli would like to acknowledge the scholarship support provided by Queensland University of Technology. We would also like to thank Dr. Barry Wood for his help with XPS analysis.

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